

Keith Crandall  
Thomas L. Martin Professor of Biology  
Department of Integrative Biology  
Brigham Young University  
Provo, UT 84602

**Specific Questions to Consider for Review of Dr. R.R. Ramey's Report on Genetic Analysis of Preble's Meadow Jumping Mouse**

1. Please analyze the techniques used in the population and phylogenetic evaluation of *Zapus hudsonius preblei* and other taxa. Were appropriate methodologies and markers used?

Appropriate markers and methods were used. The control region would provide the highest possible resolution using mtDNA. As the authors state, microsatellites would provide additional insights but would not alter the general conclusion. Another mtDNA locus would also help support the conclusions (phylogenetic methods typically do better with longer sequences), but again would not change the basic conclusion. The analytical methods used are appropriate were performed quite well, in my opinion. The only additional analysis I might perform is to construct a network relationship of gene genealogies using TCS or SplitTree software. But once again, this simply allows for a different visualization of the same result. The conclusion will not change.

2. Are the conclusions about the taxonomic validity of *Z.h. preblei* logical and defensible as presented in the manuscript?

Indeed, the conclusions are right on. This work is particularly impressive by its inclusion of both genetic and morphometric data coupled with an evaluation of previous work. The author is spot on in every respect. Indeed, it looks like you will have some more work to go to figure out an appropriate taxonomy for this group. The current taxonomy clearly does not reflect the inferred evolutionary relationships. But it is clear that the *Z. H. preblei* is not a valid taxon and that the animals on the front range of CO are genetically represented in other areas.

3. Are there possible alternative interpretations of the genetics data?

I can't think of any – at least not relative to the taxonomic status of the Preble's Meadow Jumping Mouse. Some additional work could be done to develop a reasonable taxonomy and make global inferences about population structure, bottlenecks, range expansions, etc. for the species.

4. Are there additional or divergent taxonomic conclusions that could be drawn from the genetics data?

I think additional taxonomic conclusions will require additional sampling. Certainly, at the moment, I would say you have two taxa here corresponding to the two clades.

5. Do you agree with the interpretation about possible mechanisms of reduced gene flow between *Z.h. preblei* and other subspecies of *Z. hudsonius*?

Yes. The interpretations could be further substantiated by additional samples and performing a Nested Clade Analysis to partition historical demographic events from current population structure and ongoing gene flow (Templeton, 1998; Templeton, 2004).

6. Do you agree with the concepts of Crandall et al. (2000)\* for defining evolutionarily significant units?

I have to say I do! I quite like that paper, as do many other folks. We have received a lot of positive feedback from it and no negative feedback that I have seen.

7. Are there clear ecological distinctions between *Z. h. preblei* and closely related taxa that would suggest a need for specific conservation actions for this taxon?

The morphological analysis suggests that there are not. If there were clear ecological differences that were persistent over evolutionary time and adaptively important, one might expect the evolution of morphological differences. In many cases, this occurs long before divergence of neutral genetic markers. For example, Polar Bears are obviously morphological distinct from Brown Bears, yet genetically they do not form distinct clades. Here we see no obvious morphological distinctiveness that relates to the designated subspecies. Indeed, the critical review of the previous work designating this subspecies identifies a number of significant problems with it. There is always a possibility that we are simply not looking at the right (critical) character. But of those examined, there does not appear to be any distinction.

In summary, I found this to be an excellent study covering all the appropriate bases. The conclusions drawn are, in my opinion, well founded and well supported by the data. The investigator has done an exceptional job in planning the study, selecting appropriate data to collect, collecting data, analyzing data, and interpreting the results. I agree with the conclusions provided by the investigator in this report and find them based on solid science.

- \* Crandall, K. A., Bininda-Emonds, O. R. P., Mace, G. M. and Wayne, R. K. 2000. Considering evolutionary processes in conservation biology: returning to the original meaning of “evolutionary significant units”. *Trends in Ecology and Evolution*: 15(7):290-295.

Templeton AR (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* 7, 381-397.

Templeton AR (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. *Molecular Ecology* 13, 789-810.